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Fluorometric Assay of the Arylsulphatases in Human Urine

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Summary: A method is described which differentiates arylsulphatase A and arylsulphatase B in human urine. 4-Methylumbelliferyl sulphate serves as the substrate, and silver ions are used to inhibit arylsulphatase A activity. Using fresh urine it is possible to obtain separate values for arylsulphatase A and B when both are present, thus providing a diagnostic test for metachromatic leucodystrophy.

Fluorimetrische Bestimmung der Arylsulfatasen im menschlichen Urin

Zusammenfassung: Für die gleichzeitige Bestimmung von Arylsulfatase A und B im Urin wurde eine empfindliche fluorimetrische Methode entwickelt, die sich des 4-Methylumbelliferylsulfates bedient. Sie stützt sich auf den unterschiedlichen Effekt von Silberionen auf die beiden Enzyme und ermöglicht die Differenzierung zwischen Normalfällen und solchen mit metachromatischer Leukodystrophie.

Introduction

Although physiological substrates for arylsulphatases A and B are known (1,2), the activity of these enzymes is usually measured colorimetrically using 4-nitrocatechol sulphate as substrate (6). These enzymes are of clinical interest, because arylsulphatase A is deficient in metachromatic leucodystrophy (3), and arylsulphatase B is deficient in mucopolysaccharidosis type VI (4,5). In an earlier report (7) 4-methylumbelliferyl sulphate was used as a sulphatase substrate in human liver. It was possible to specifically inhibit arylsulphatase A activity with Ag^+ ions. *Christomanou & Sandhoff* (8) used the same method to differentiate the sulphatases in isolated human leucocytes.

The present communication describes the application of these principles to the simple and rapid assay of sulphatases A and B in urine.

Materials and Methods

4-Methylumbelliferyl sulphate was obtained from Koch-Light, 4-nitrocatechol sulphate from Sigma, and $AgNO_3$ from Merck.

Enzyme assay

2.5 ml fresh urine was added to PD-10 columns (Sephadex G-25, 1.5 × 5 cm, bed volume 9 ml; Pharmacia), equilibrated with 50 mmol/l Tris-HCl buffer pH 7.4, and the effluent was discarded. The high molecular weight components, e.g. enzyme proteins, were eluted with 3.5 ml 50 mmol/l Tris-HCl buffer pH 7.4 and concentrated to about 300 µl using minicon-A 25 (Amicon). The enzyme assay was performed as described previously (7). 100 µl of 5 mmol/l 4-methylumbelliferyl sulphate in redistilled water, 25 µl of 1 mmol/l acetate buffer, pH 5.5, and 25 µl of prepared urine sample were incubated for 30 min. The reaction was stopped by addition of 3.0 ml of 0.2 mol/l glycine buffer, pH 10.7. The fluorescence was immediately read in an Aminco-Bowman spectrophotofluorimeter with excitation wave-length 365 nm and emission wave-length 448 nm. 4-Methylumbelliferone in glycine buffer was used as a standard.

Ion-exchange chromatography

500 ml fresh urine were concentrated to about 12 ml using polyethylene glycol. The concentrated urine was dialysed overnight against 50 mmol/l Tris-HCl pH 7.4, centrifuged (1000 *g*, 10 min), then applied to the column. The column contained preswollen DEAE-cellulose (column 0.9 × 24 cm) equilibrated in 50 mmol/l Tris-HCl buffer, pH 7.4. After elution with 65 ml of 50 mmol/l Tris-HCl buffer, pH 7.4, a linear gradient was established with 100 ml of this buffer and 100 ml of the same buffer containing 0.7 mol/l of NaCl. 1 ml fractions were collected with a flow rate of 40 ml per hour.

Results

A typical pH profile found in fresh urine specimen prepared as described in Materials and Methods is given in figure 1. The pH profile after storage of urine samples at −20 °C or 4 °C (1–7 days) is also given in figure 1.

The pH optimum for sulphatase activity in most of the fresh urine samples was around pH 5.5 while in the same stored urine samples the pH optimum was shifted towards pH 6.0. In a few fresh urines (15 %), the

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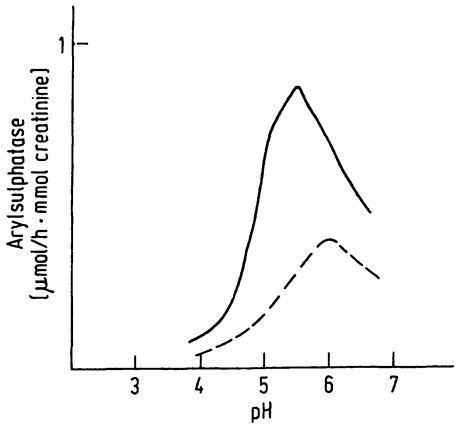

Fig. 1. pH dependence in fresh, prepared urine (——) and in the same urine stored 24 hours (or 2–7 days) at 4 °C or –20 °C before preparation (– – – –). 1 mol/l acetate buffer was used. Activity is given as μmol substrate split per hour and per mmol creatinine.

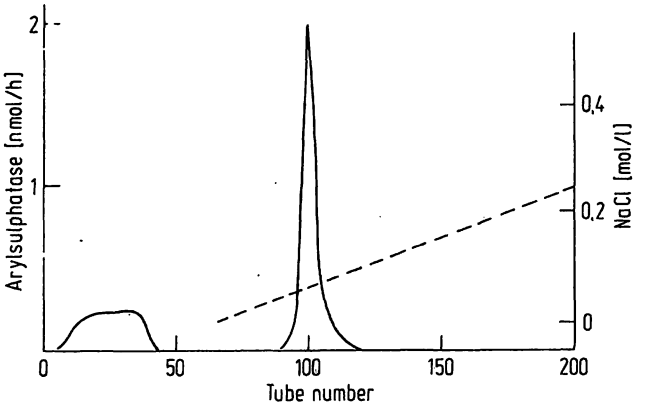

Fig. 2. Ion-exchange chromatography of urine. Preparation of 500 ml urine is described in Materials and Methods. Activity is given as nmol substrate split per hour under the specified assay conditions (see Materials and Methods and l.c. (7)). Activity towards 4-methylumbelliferyl sulphate (——). Sodium chloride gradient (– – – –).

sulphatase activity was approximately constant in the range pH 5.5–6.5. The activity especially at and below pH 5.5 was strongly reduced (more than 50 %) after storage for only 24 hours. It is not known whether there was any further inactivation of the enzyme during the 7 days of storage.

Arylsulphatase B normally exhibited 18–64 % (mean 35 %) of the total sulphatase activity. When 20 consecutive assays were made on the same urine, a coefficient of variation of 5 % was obtained for arylsulphatase A and B.

There was no correlation between arylsulphatase activity and creatinine content of the urine. The correlation coefficient was –0.112 for arylsulphatase A and 0.131 for arylsulphatase B (20 urine samples were tested).

The activity level of arylsulphatases A and B in 20 controls and three patients with metachromatic leucodystrophy is given in table 1.

Figure 2 shows the sulphatase pattern found after chromatography. Activity towards 4-methylumbelliferyl sulphate showed one unadsorbed peak and one adsorbed peak. Assay of arylsulphatases A and B according to *Baum* et al. (6) showed that arylsulphatase B activity is found in the unadsorbed peak and arylsulphatase A activity in the adsorbed peak.

Tab. 1. Arylsulphatases A and B in urine from twenty controls and three patients with metachromatic leucodystrophy (MLD). The activities are expressed as μmol of 4-methylumbelliferone released per hour and per mmol creatinine at pH 5.5.

	Arylsulphatase A	Arylsulphatase B
Controls (n = 20)	0.2–1.5	0.06–0.64
MLD patient 1	0.04	0.20
MLD patient 2	0.02	0.36
MLD patient 3	0.02	0.40

The pH optimum of both peaks, using 4-methylumbelliferyl sulphate, was pH 5.5. The enzyme activity was proportional to time at pH 5.5 for at least 60 min. The apparent *Michaelis-Menten* constant was 1.6 mmol/l for arylsulphatase A and 0.5 mmol/l for arylsulphatase B (1 mol/l acetate buffer, pH 5.5). In the presence of $AgNO_3$ (range 0.003–2 mmol/l) arylsulphatase A was inhibited by about 96–98%, while arylsulphatase B was unaffected. At a concentration of 0.001 mmol/l $AgNO_3$, arylsulphatase A was only inhibited by 50 %. The pH optima of the two arylsulphatases were not significantly altered by $AgNO_3$.

Discussion

The sulphatase pattern found in ion-exchange chromatography indicates that urine exhibits very little, if any, activity of arylsulphatase C (which is present in human liver and is eluted around fraction 120–130, as described in l.c. (7)). Arylsulphatase A is adsorbed and arylsulphatase B unadsorbed, as described for the enzymes from human liver (7). pH optimum and inhibition of arylsulphatase A with Ag^+ ions is also in agreement with earlier reports (7,8).

The strong decrease of activity during storage, especially at and below pH 5.5, was accompanied by a shift of the pH optimum towards pH 6.0. This might indicate the presence of some activity of arylsulphatase C which then should be more resistant to storage. The existence of arylsulphatase C could also explain the constant activity between pH 5.5 and 6.5, which was found in a few of the fresh urine samples.

It is important to point out that the sulphatase assay should be performed on fresh urine, because storage strongly reduces the enzyme levels even after only 24 hours.

Arylsulphatase B exhibited 18–64 % of the total sulphatase activity in urine. This is in agreement with

earlier results using 4-nitrocatechol sulphate as substrate (9). By measuring arylsulphatase at pH 5.5 in fresh urine, in the presence and absence of silver ions, it is possible to obtain separate values for arylsulphatase A and B, and thus diagnose metachromatic leucodystrophy.

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References

1. Mehl, E. & Jatzkewitz, H. (1968), Biochim. Biophys. Acta *151*, 619–627.
2. Matalon, R., Arbogart, B. & Dorfman, A. (1974), Biochem. Biophys. Res. Commun. *61*, 1450–1457.
3. Austin, I., McAfee, D. & Shearer, L. (1965), Arch. Neurol. *12*, 447–455.
4. Beratis, N.-G., Turner, B. M., Weiss, R. & Hirschhorn, K. (1975), Pediatr. Res. *9*, 475–480.
5. Fluharty, A. L., Stevens, R. L., Fung, D., Peak, S. & Kihara, H. (1975), Biochem. Biophys. Res. Commun. *64*, 955–961.
6. Baum, H., Dodgson, K. S. & Spencer, B. (1959), Clin. Chim. Acta *4*, 453–455.
7. Hultberg, B. (1977), Clin. Chim. Acta *80*, 423–430.
8. Christomanou, H. & Sandhoff, K. (1977), Clin. Chim. Acta *79*, 527–531.
9. Hagberg, B. & Öckerman, P. A. (1970), Neuropädiatrie *2*, 53–58.

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